	SampleName	Inj. Volume	Channel	Dilution
1	K63 in PBS	100,00	214nm	4,00
2	K63 in Chaps 0,25%	100,00	214nm	4,00
3	K63 in citrate	100,00	214nm	4,00

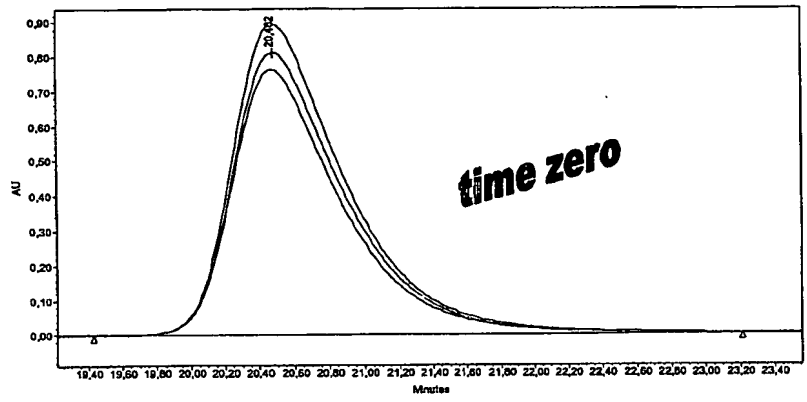


Figure 1A

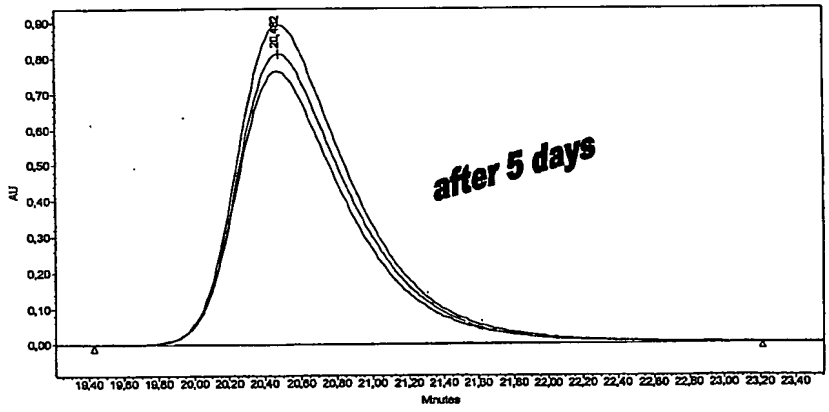


Figure 1B

Figure 1C

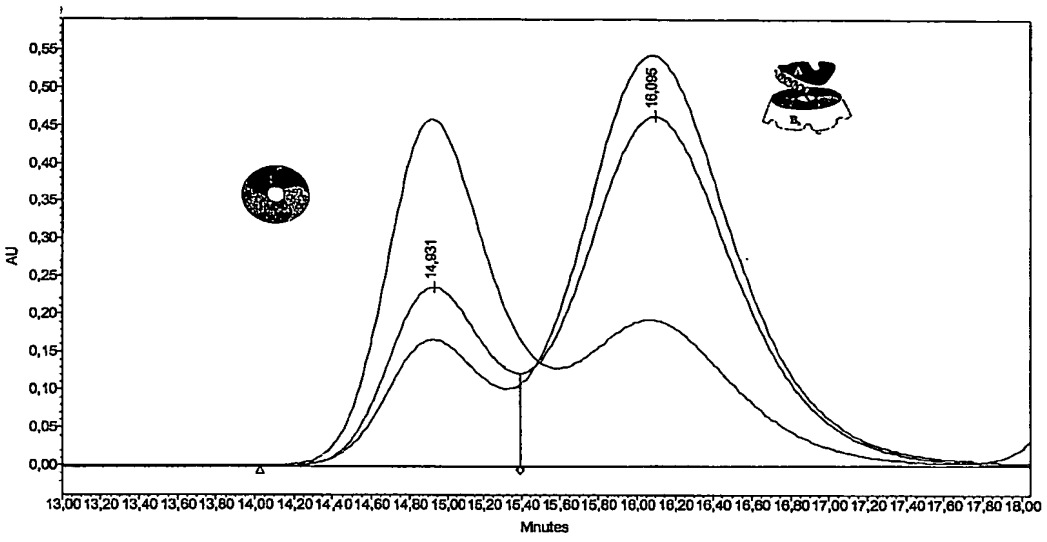
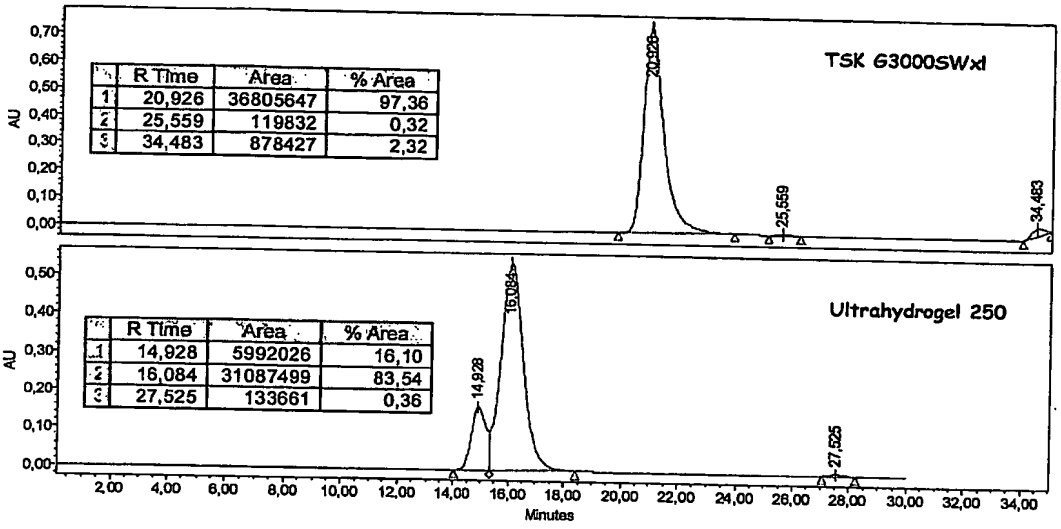
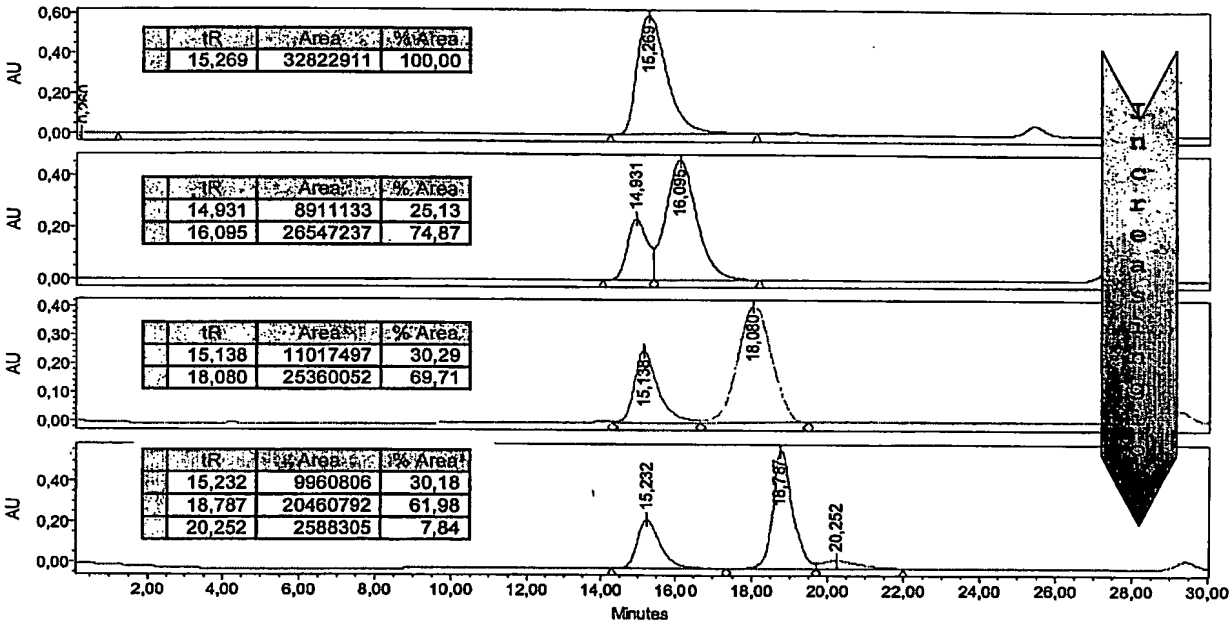

AU
0,55
0,50
0,45
0,40
0,35
0,30
0,25
0,20
0,15
0,10
0,05
0,00
14,931
16,095
13,00 13,20 13,40 13,60 13,80 14,00 14,20 14,40 14,60 14,80 15,00 15,20 15,40 15,60 15,80 16,00 16,20 16,40 16,60 16,80 17,00 17,20 17,40 17,60 17,80 18,00
Minutes

Figure 1D


TSK G3000SWxl
R Time | Area | % Area
1 | 20,926 | 36805647 | 97,36
2 | 25,559 | 119832 | 0,32
3 | 34,483 | 878427 | 2,32
20,926
25,559
34,483
Ultrahydrogel 250
R Time | Area | % Area
1 | 14,928 | 5992026 | 16,10
2 | 16,084 | 31087499 | 83,54
3 | 27,525 | 133661 | 0,36
14,928
16,084
27,525
AU
Minutes

Figures 2A-2D

	SampleName	Date Acquired	Eluent	Injection Volume	Channel	Dilution
1	PBS 5gg agitazione	09/04/2003 9.55.19	KPI 50 mM + Na2SO4 50 mM pH 7,2	100,00	214nm	4,00
2	PBS 5gg agitazione	08/04/2003 13.53.06	KPI 100 mM + Na2SO4 100 mM pH 7,2	100,00	214nm	4,00
3	PBS 5gg agitazione	09/04/2003 15.07.11	KPI 250 mM + Na2SO4 100 mM pH 7,2	100,00	214nm	4,00
4	PBS 5gg agitazione	10/04/2003 9.51.42	KPI 200 mM + Na2SO4 200 mM pH 7,2	100,00	214nm	4,00



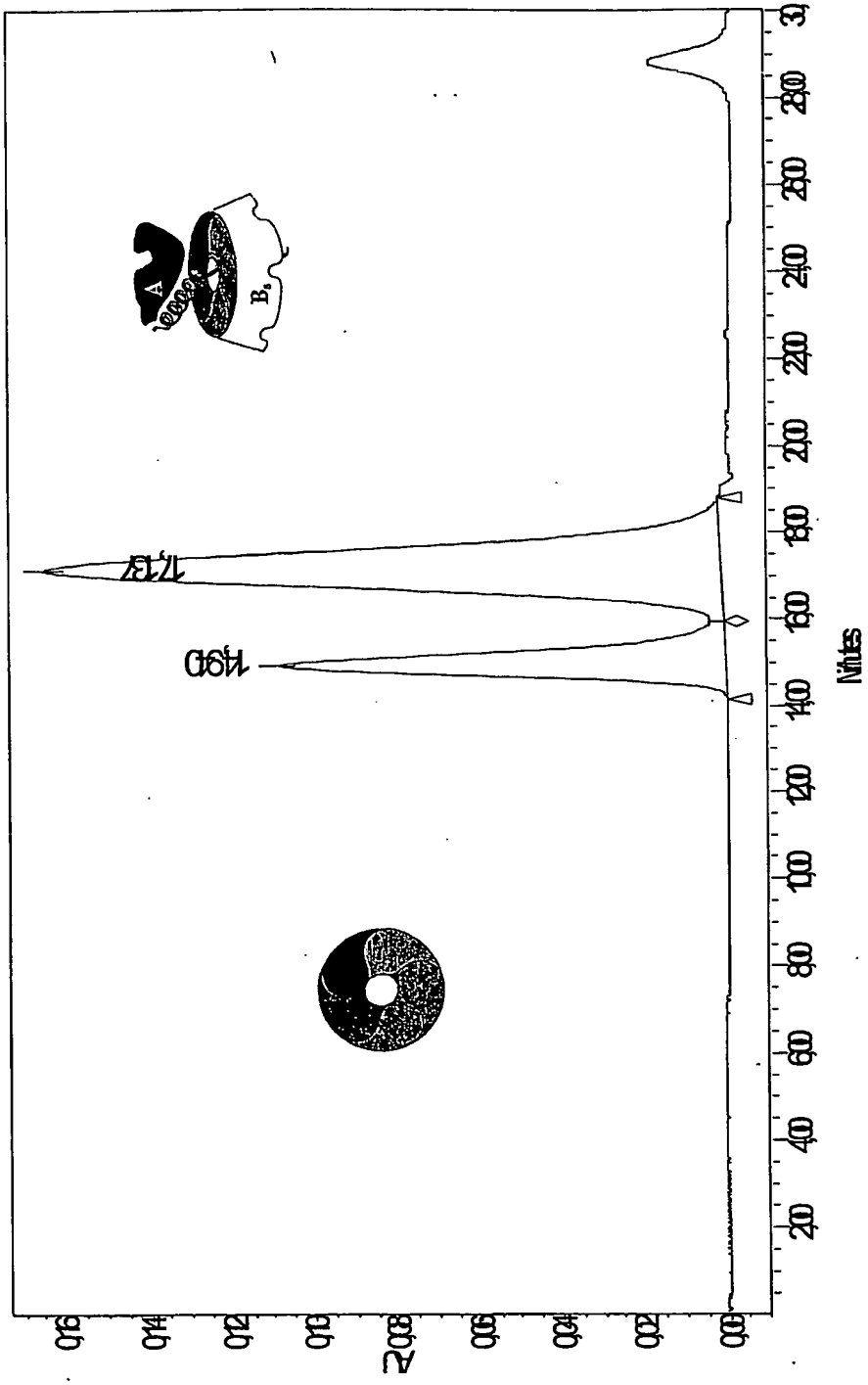


Figure 3

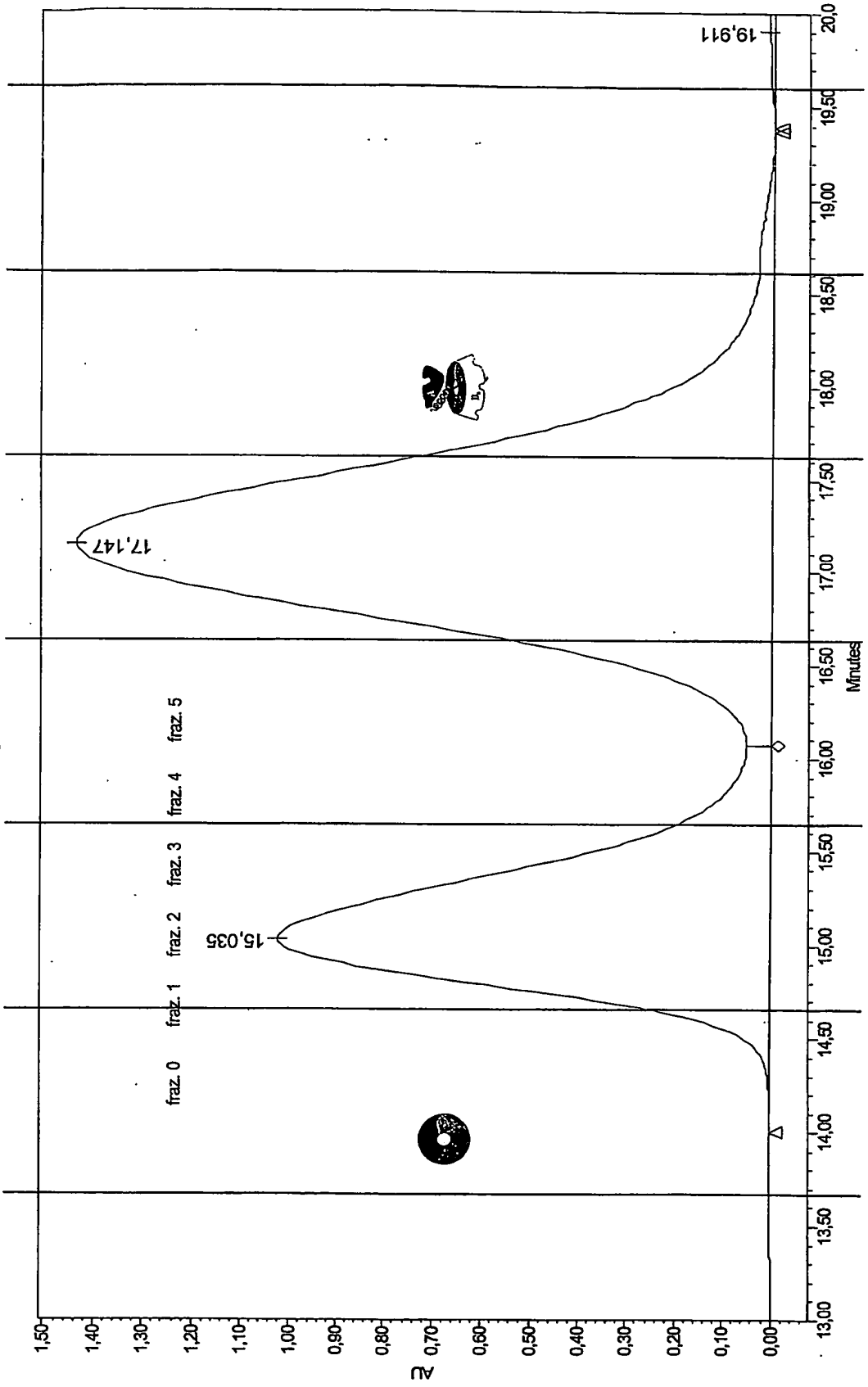

19,911
17,147
15,035
fraz. 0
fraz. 1
fraz. 2
fraz. 3
fraz. 4
fraz. 5
AU
Minutes
1,50
1,40
1,30
1,20
1,10
1,00
0,90
0,80
0,70
0,60
0,50
0,40
0,30
0,20
0,10
0,00
13,00
13,50
14,00
14,50
15,00
15,50
16,00
16,50
17,00
17,50
18,00
18,50
19,00
19,50
20,0

Figure 4

Figure 5A

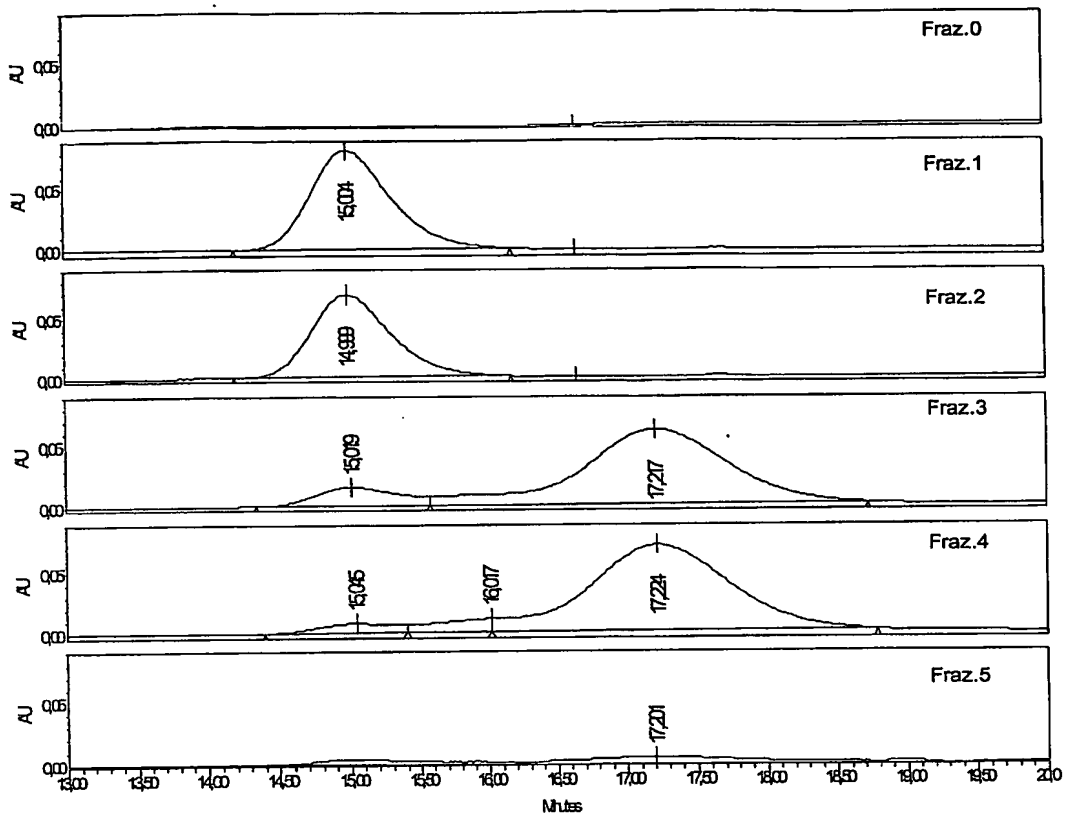


Figure 5B

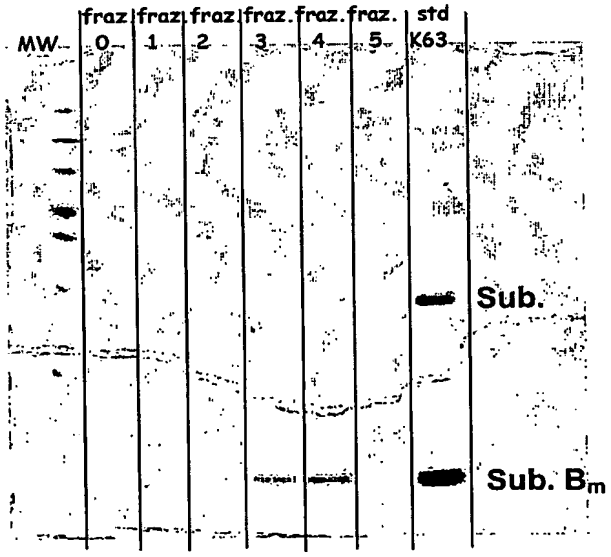


Figure 5C

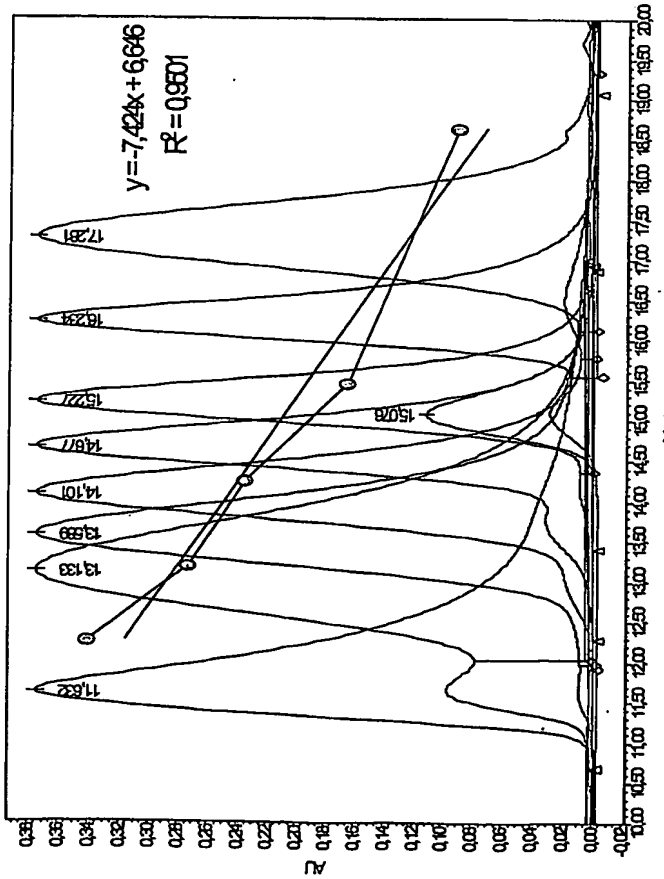


Figure 5D

Standard proteins	Rt (min)	M_w (Da)
Thyroglobulin (bovine)	11,62	669.000
Apoferritin	13,13	476.316
B-amylase	13,58	224.340
Alcohol Deydrogenase	14,10	146.980
BSA	14,67	66.800
Carbonic Anhydrase	16,22	29.023
Sample proteins	**Rt (min)**	**M_w exp.**
CRM	15,23	57.099
K63 AB_5	17,26	9.611
K63 B_5	15,07	65.607

Superimposition of standard proteins, CRM_{197} reference (bold blue), K63 (bold red) an calibration curve used for apparent MW determination.

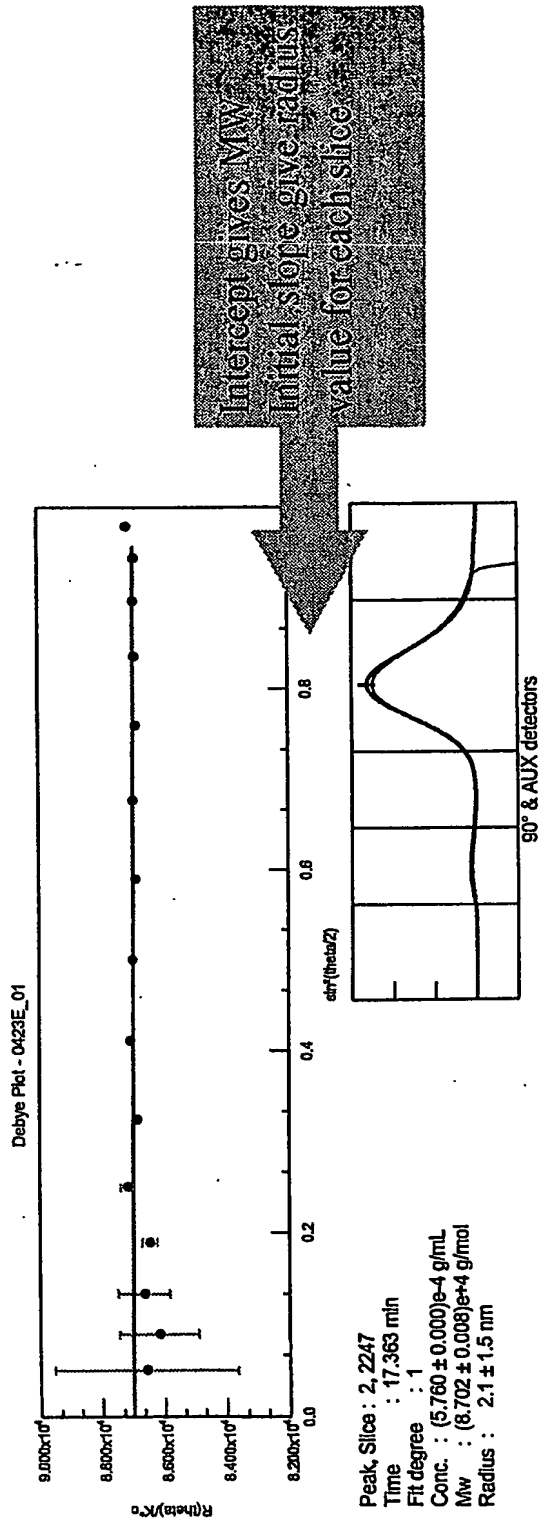

Intercept gives MW
Initial slope give radius
value for each slice
Debye Plot - 0423E_01
R(theta)/Kc
9.000x10⁴
8.800x10⁴
8.600x10⁴
8.400x10⁴
8.200x10⁴
0.0
0.2
0.4
0.6
0.8
dn/d(theta/2)
90° & AUX detectors
Peak, Slice : 2, 2247
Time : 17.363 min
Fit degree : 1
Conc. : (5.760 ± 0.000)e-4 g/mL
Mw : (8.702 ± 0.008)e+4 g/mol
Radius : 2.1 ± 1.5 nm

Figure 5E

Figure 5F(b)

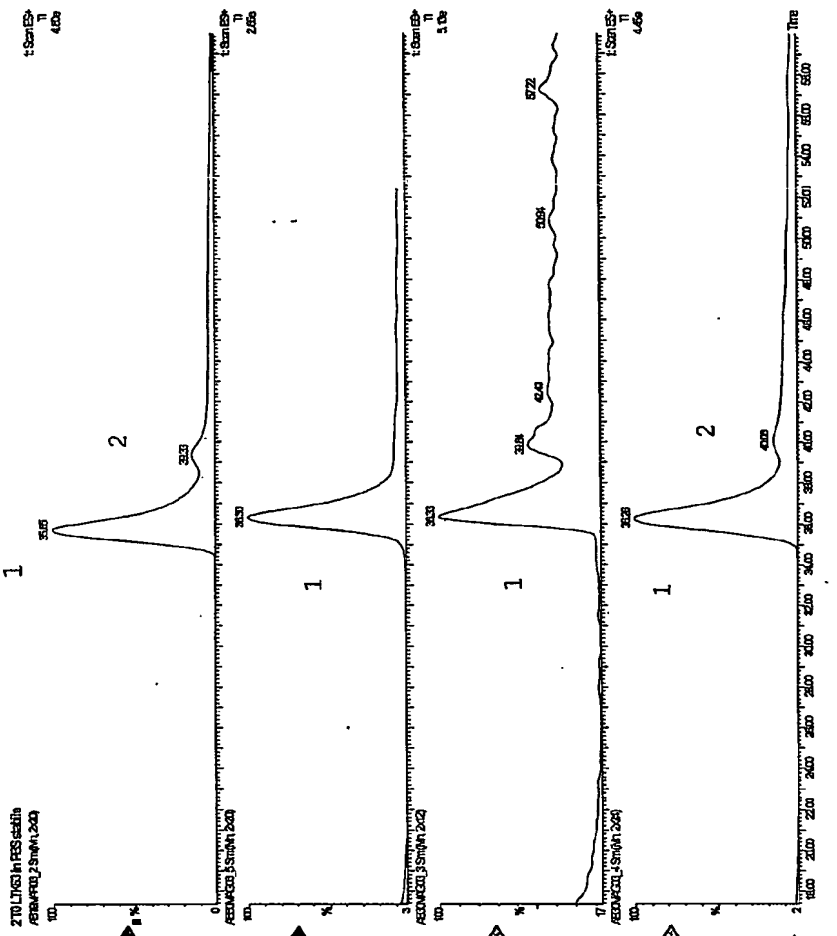


Figure 5F (a)

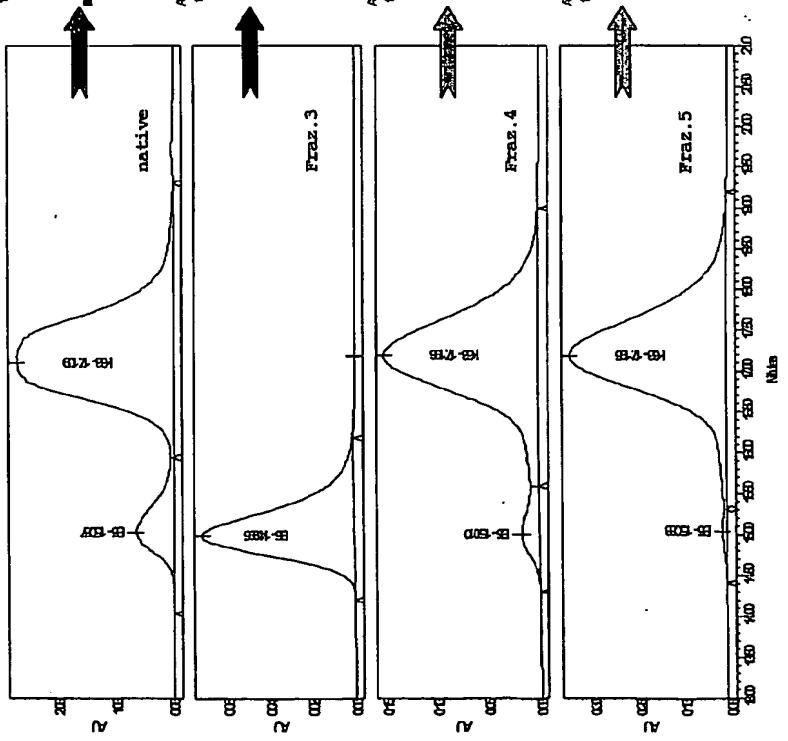


Figure 5G

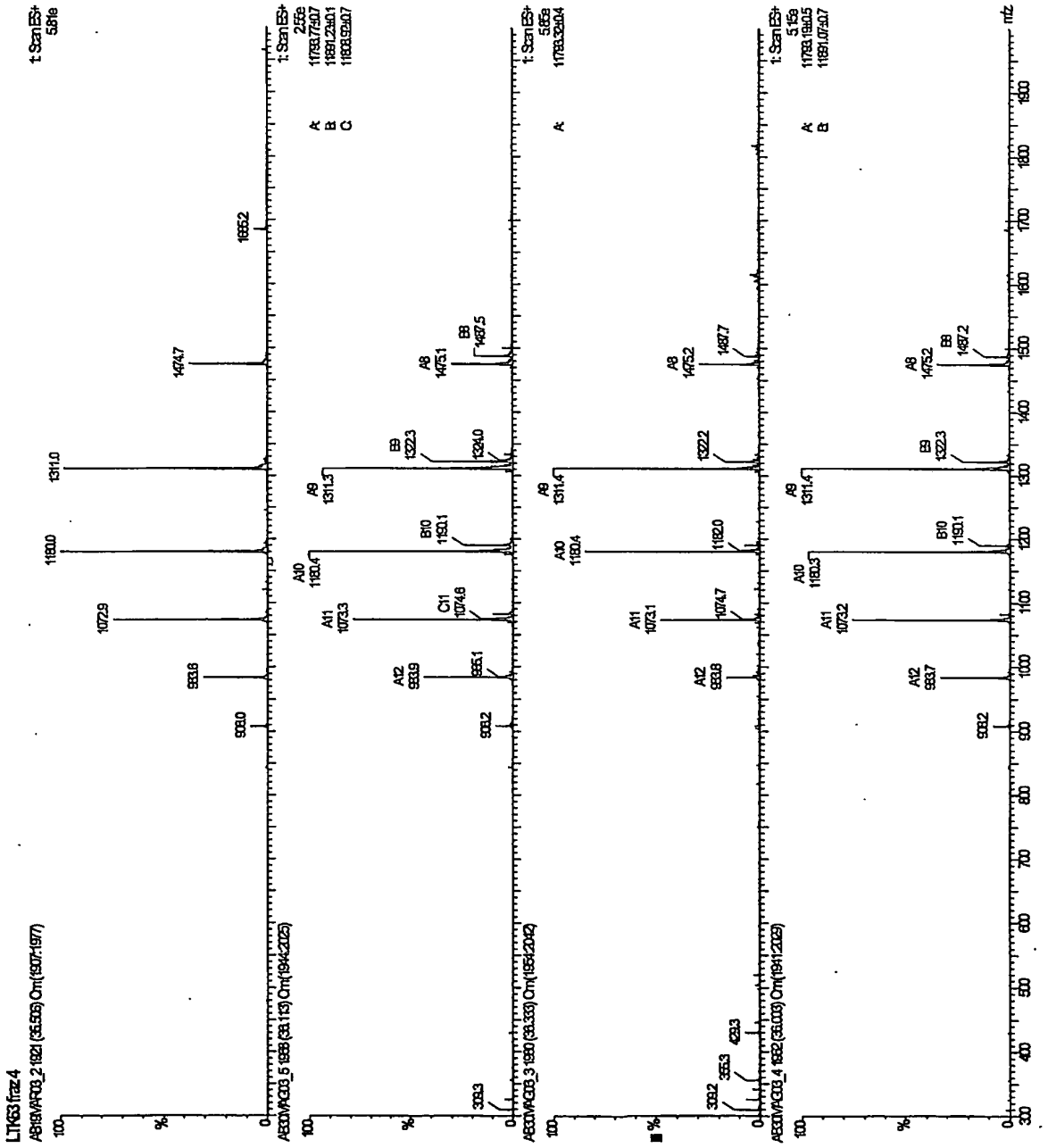


Figure 6: SDS-PAGE analysis of LTK 63 shaken samples

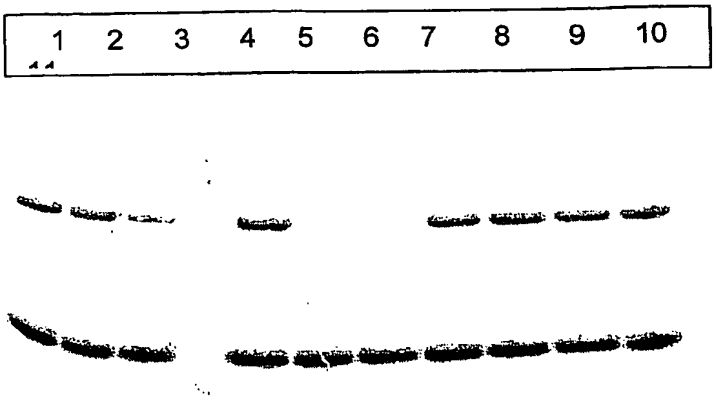
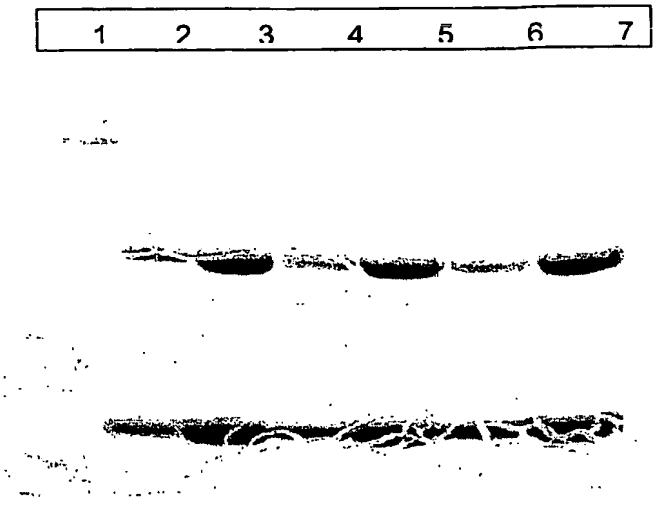
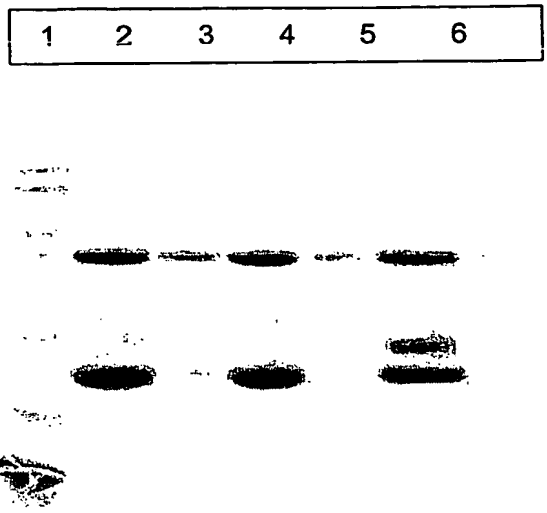


Figure 7: SDS-PAGE analysis of LTK 63 samples treated with CHAPS



1 MW
2 LTK63 in phosphate 20 mM, CHAPS 0.1% supernatant T3
3 LTK63 in phosphate 20 mM, CHAPS 0.1% pellet T3
4 LTK63 in phosphate 20 mM, CHAPS 0.25% supernatant T3
5 LTK63 in phosphate 20 mM, CHAPS 0.25% pellet T3
6 LTK63 in phosphate 20 mM, CHAPS 0.5% supernatant T3
7 LTK63 in phosphate 20 mM, CHAPS 0.5% pellet T3

Figure 8: SDS-PAGE of LTK63 samples treated with L-Arginine



1 MW
2 LTK63 2 mg/ml in phosphate 20 mM, L-arginine 100 mM supernatant T1
3 LTK63 2 mg/ml in phosphate 20 mM, L-arginine 100 mM pellet T1
4 LTK63 4 mg/ml in phosphate 20 mM, L-arginine 100 mM supernatant T1
5 LTK63 4 mg/ml in phosphate 20 mM, L-arginine 100 mM pellet T1

Figure 9(a): Old HPLC Method for analysing L-Arginine treated samples

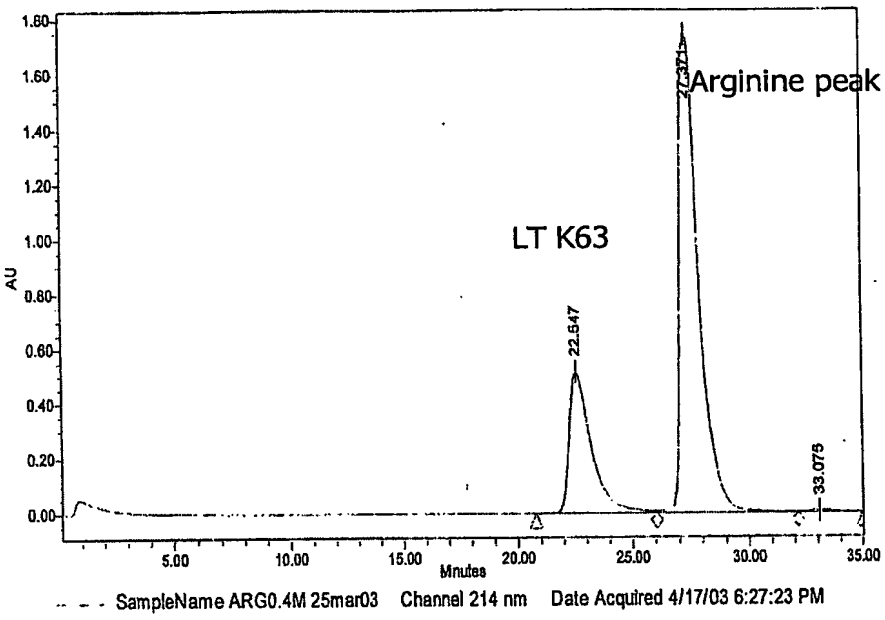


Figure 9(b): New HPLC Method for analysing L-Arginine treated samples

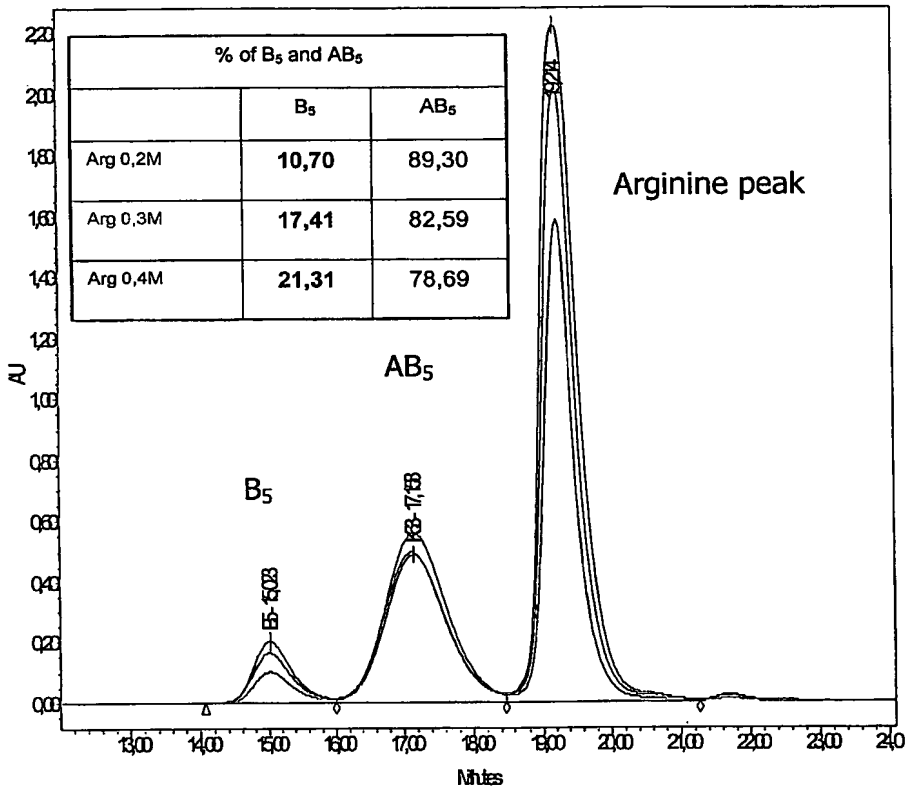


Figure 10(a): Determination of AB5 dissociation in L-Arginine treated samples and the %B5 in LTK63 at 1.3mg/ml

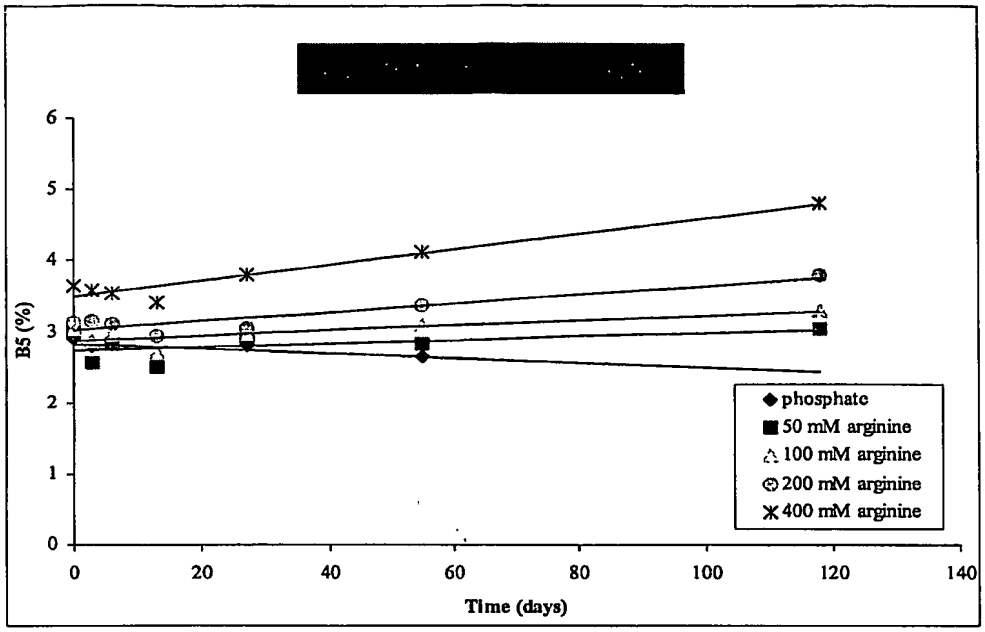


Figure 10(b): Determination of AB5 dissociation in L-Arginine treated samples and the %B5 in LTK63 at 4.0mg/ml

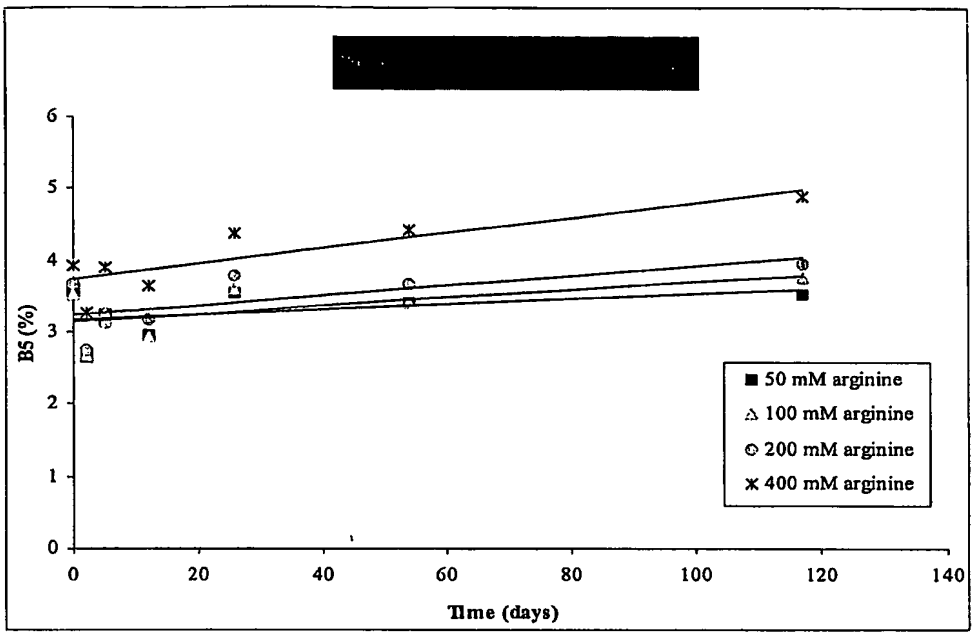


Figure 11(a): CHAPS effect on LTK63 dissociation

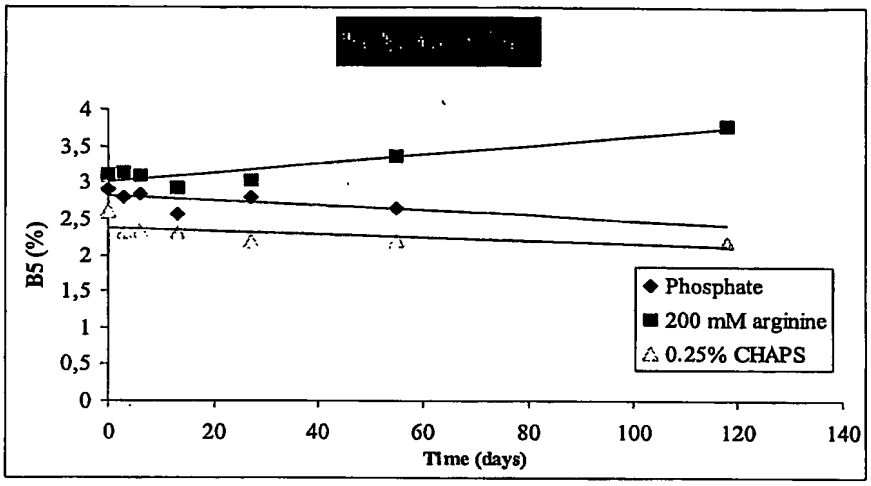


Figure 11(b): CHAPS effect on LT K63 dissociation in combination with L-Arginine

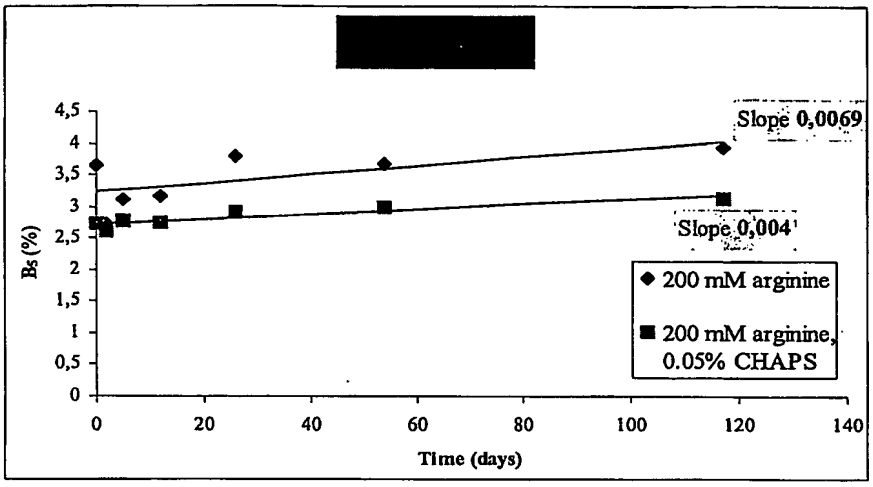


Figure 12: Effect of L-Arginine and CHAPS on LTK 63 stability at a protein concentration of 1,3 mg/ml

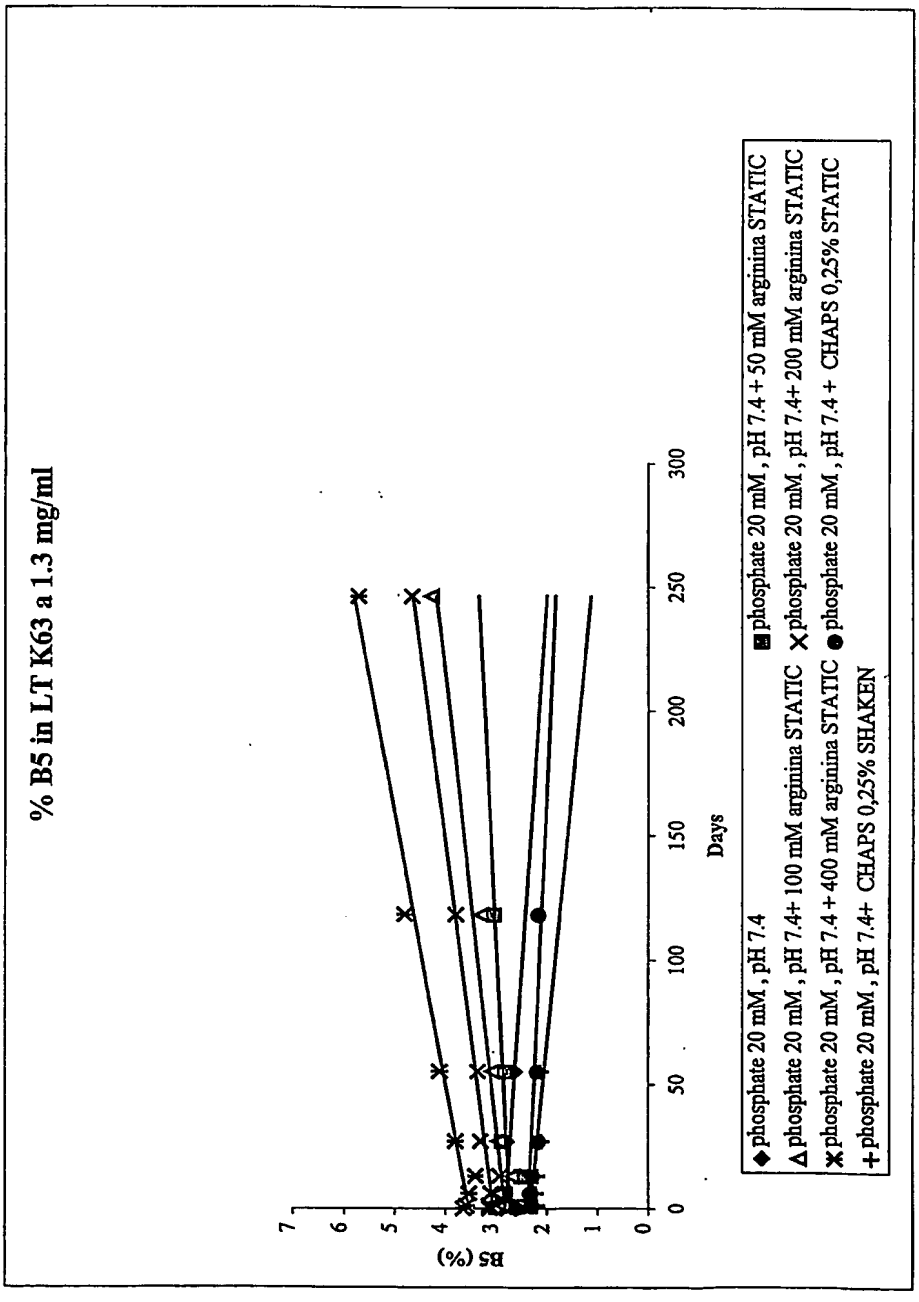


Figure 13: The effect of L-Arginine and the combination L-Arginine/CHAPS on LTK 63 stability at a protein concentration of 4,0 mg/ml

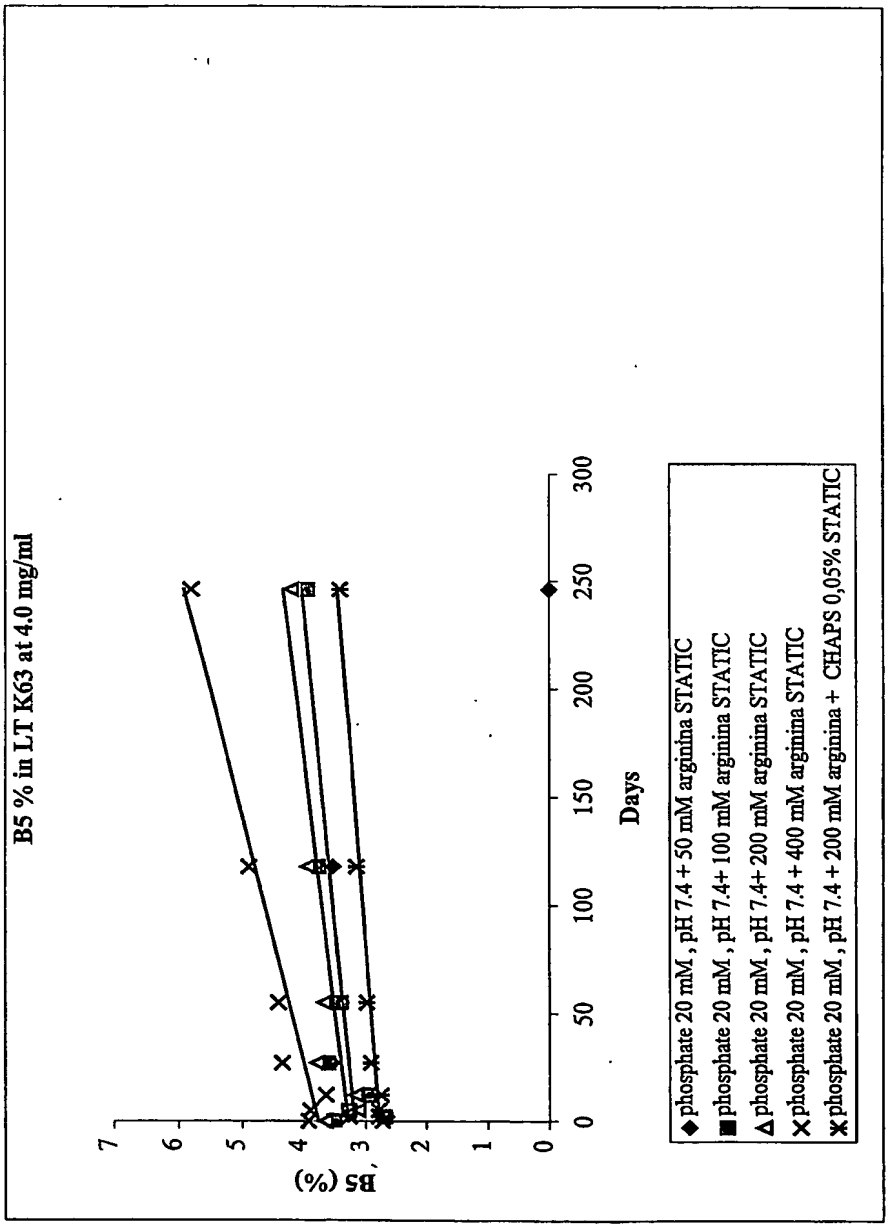
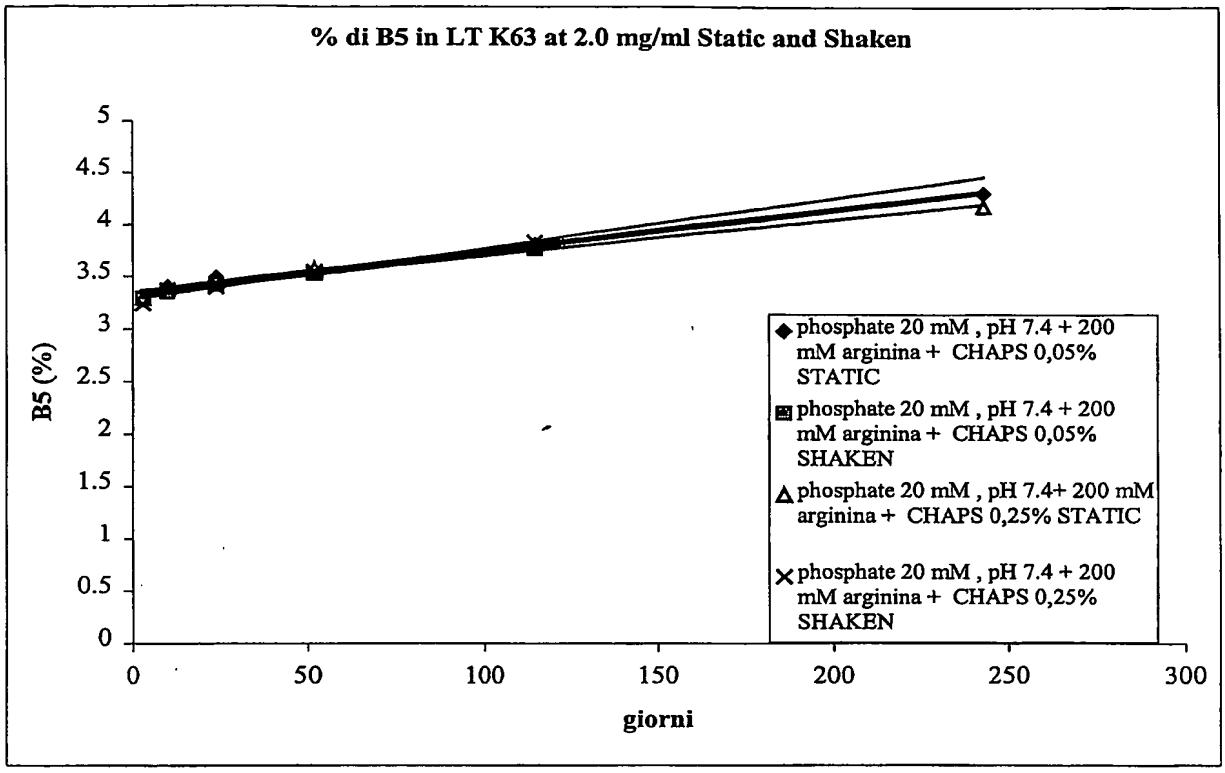


Figure 14 shows the effect of storage conditions on LTK 63 stability in L-Arginine + CHAPS containing buffers



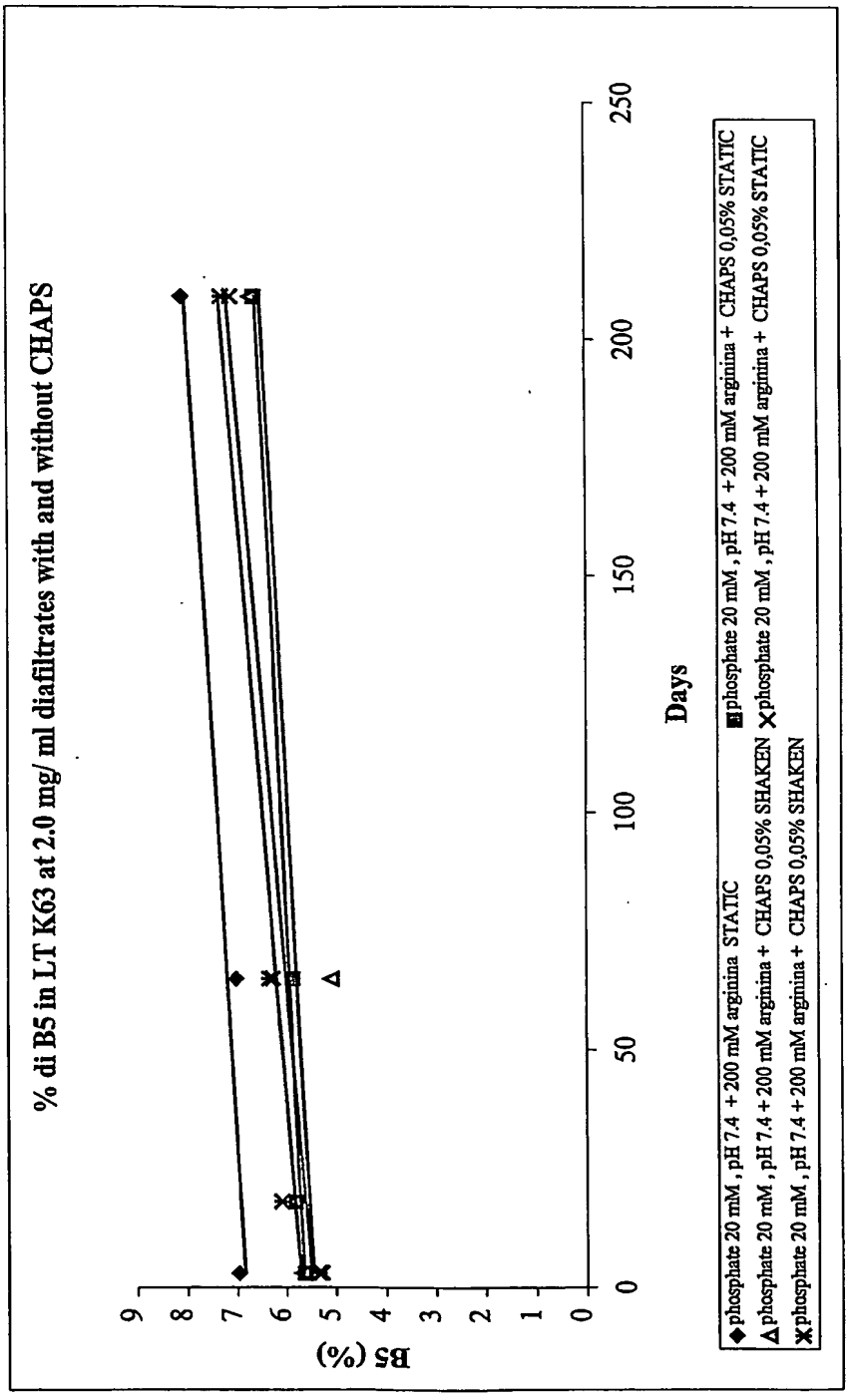


Figure 15: Comparison of LTK 63 stability on L-Arginine and L-Arginine + CHAPS storage buffers

Figure 16

Table 2. Structure and Classification of Detergents (continued)

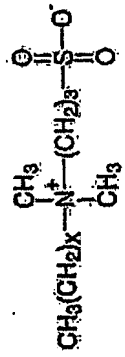
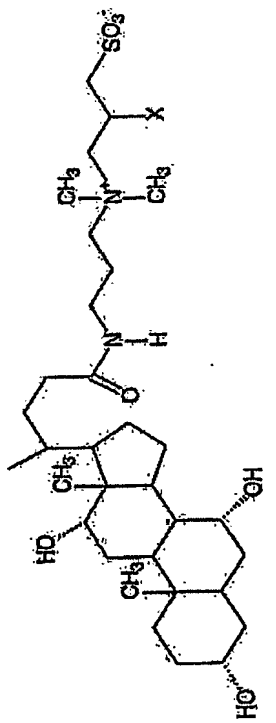
Zwittergents		EMPIGEN BB® (*n*-dodecyl-*N*,*N*-dimethylglycine)
		x = 7, ZWITTERGENT® 3-08 x = 9, ZWITTERGENT® 3-10 x = 11, ZWITTERGENT® 3-12 x = 13, ZWITTERGENT® 3-14 x = 15, ZWITTERGENT® 3-16
		x = H, CHAPS x = OH, CHAPSO

Figure 17

Zwitterionic Detergents

Product	Cat. No.	M.W. (anhydrous	CMC[b] (mM)	Aggregation No.	Average Micellar Weight	size
ASB-14	182750	434.7	—	—	—	5g,25 g
ASB-16	182755	462.7	—	—	—	5g,25 g
CHAPS	220201	614.9	6-10	4-14	6000	1 g 5 g 10 g 25 g
CHAPSO	220202	630.9	8	11	7000	1 g 5 g
DDMAB	252000	299.5	4.3	—	—	5 g
DDMAO	252005	339.6	0.13	—	—	5 g
EMPIGEN BB® Detergent, 30% Solution	324690	272.0	1.6-2.1	—	—	100 ml
Lauryldimethylamine Oxide (LDAO), 30% Solution	428011	229.4	1-2	76	17,000	100 ml
ZWITTERGENT® 3-08 Detergent	693019	279.6	330	—	—	5 g
ZWITTERGENT® 3-10 Detergent	693021	307.6	25-40	41	12,500	5 g 25 g
ZWITTERGENT® 3-12 Detergent	693015	335.6	2-4	55	18,500	5 g 25 g
ZWITTERGENT® 3-14 Detergent	693017	363.6	0.1-0.4	283	30,000	5 g 25 g 100 g
ZWITTERGENT® 3-16 Detergent	693023	391.6	0.01-0.06	155	60,000	5 g 25 g

a. Average molecular weights are given for detergents composed of mixtures of chain lengths; b. Temperature: 20 - 25°C

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